EXHIBIT 49

Persistent *Acinetobacter baumannii*? Look Inside Your Medical Equipment

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ABSTRACT

Two outbreaks of multidrug-resistant *Acinetobacter baumannii* occurred in our hospital. The outbreak strains were eventually isolated from respiratory ventilators, an apparatus used to cool or warm patients, and four continuous veno-venous hemofiltration machines. Removing dust from the machines and replacing all dust filters brought the outbreaks to an end (*Infect Control Hosp Epidemiol* 2004;25:1002-1004).

Multidrug-resistant *Acinetobacter baumannii* continues to cause outbreaks in intensive care units (ICUs) around the world.[1-3] The epidemiology of *A. baumannii* is diverse. Outbreaks may originate from a single contaminated source such as ventilators or room humidifiers, the outbreak strain may become widespread with heavy contamination of the environment, or no reservoir may be found.[1-5]

Between October 2000 and July 2003, two outbreaks caused by two distinct strains of multidrug-resistant *A. baumannii* occurred in our university hospital. Both outbreaks occurred in the medical ICU and spread to other ICUs located in different parts of the hospital.

The first outbreak began in October 2000 and was caused by a multidrug-resistant strain of *A. baumannii*. In total, 29 patients were affected, 27 being colonized and 2 having a possible infection (positive blood cultures). All 29 patients were colonized in the respiratory tract. In 17 patients, the respiratory tract was the body site found to be colonized first. Colonized patients were placed in strict isolation (ie, placed in a separate room with an anteroom with negative air pressure). In addition, healthcare workers entering the patients' rooms wore protective clothing, gloves, and a mask at all times. Hygienic measures were intensified (ie, healthcare workers were instructed about strict adherence to hand hygiene between and during bronchial washings, flushing gastric tubes, caring for wounds, and washing of patients). Supplies of utility goods at the patients' bedsides were kept to a minimum. The cleaning of reusable items such as ventilation bags and tubing was improved by placing them appropriately in the washing machine. Hammocks for weighing patients were adequately cleaned and disinfected. Also, the ward was cleaned more frequently, with particular attention paid to areas where dust was likely to gather.

Although environmental cultures showed no contamination of objects on the ward, the medical ICU was at one time closed and cleaned extensively. However, soon after the ward was reopened, the outbreak strain was again isolated from patients. A second investigation into a possible environmental source was begun.

The second outbreak of a multidrug-resistant strain of *A. baumannii* began in May 2003 in the medical ICU. One patient had an infection of a hip prosthesis by *A. baumannii* superimposed on an infection caused by *Staphylococcus aureus*. Three other patients became colonized with the multidrug-resistant strain, two in the respiratory tract and one in the digestive tract. When a patient in the neurosurgery ICU who had not been admitted to the medical ICU became colonized with the same strain in an abdominal wound, an investigation was started. The findings of the investigation of the first outbreak were helpful in tracing the source of the second outbreak quickly.

METHODS

Initially during the first outbreak, 110 environmental objects were sampled including sinks, table tops, computer keyboards, objects on the crash car, ventilation masks, key panels of ventilators, monitors, infusion equipment, hammocks for weighing patients, air conditioner inlets and outlets, and dust. Medical equipment was sampled after it had been cleaned and disinfected according to standard procedures.

Later during the first outbreak, the environmental investigation consisted of sampling a so-called test lung used to check the ventilators before each new patient, a ventilator that was cleaned and disinfected according to standard protocol, and the filters inside the Bair Hugger (Augustine Medical, Inc., Eden Prairie, MN). The ventilator was sampled by opening both the pneumatic and the electronic parts and harvesting the dust from the interior. The interior of the ventilation tubing inside the pneumatic part of the ventilator was not sampled. The Bair Hugger is connected to the patient's mattress by a tube through which cold or warm air is passed to either cool or warm the patient. Several dust filters from the interior of the Bair Hugger were cultured.

Samples for cultures were collected from the surface of the objects with a moistened swab. Dust was collected using moistened swabs. Swabs were then vigorously shaken in 40 mL of acetate mineral medium for enrichment.[6] Filters were cut into small pieces and added to the mineral medium. After 48 hours in a shaking incubator at 30°C, the medium was subcultured onto sheep blood agar and cystine lactose electrolyte deficient agar. Sheep blood sedimentation plates were placed in patients' isolation rooms, on the ward, and in the nurses' station.

Isolates were identified as *Acinetobacter* and susceptibility tests were performed using VITEK 2 (bioMérieux, Hertogenbosch, the Netherlands). Species and strain identification was performed using amplified fragment-length polymorphism (AFLP).

AFLP, previously found to be a useful fingerprinting method,[7] was performed according to Nemec et al.[8] Briefly, purified DNA was digested with *Eco*RI and *Mse*I while ligation of *Eco*RI and *Mse*I adapters was performed. Polymerase chain reaction was performed with a Cy5-labeled *Eco*RI+A primer and a *Mse*I+C primer (A and C representing selective nucleotides). The ALFexpress II DNA analysis system (Amersham Biosciences, Roosendaal, the Netherlands) was used for fragment sep-

aration. Fragments of 50 to 500 bp were subjected to cluster analysis using BioNumerics software (release 2.5; Applied Maths, Sint-Martens-Latem, Belgium) with an overall tolerance setting of 0.1%. The Pearson product moment coefficient (r) was used as a measure of similarity, and the unweighted pair group average linked method was used for grouping.

RESULTS

The initial environmental investigation revealed no contaminated objects. Only one sedimentation plate from a colonized patient's isolation room within 3 m of the patient's bed was positive. During the subsequent environmental investigation of the first outbreak, the outbreak strain was isolated from medical equipment (ie, from dust in the interior of a mechanical ventilator and from filters inside the Bair Hugger). The isolates of the patients and the isolates from the ventilator and the Bair Hugger were resistant to all beta-lactam antibiotics, cotrimoxazole, and the fluoroquinolones. They were intermediately susceptible to meropenem and amikacin, and fully susceptible to tobramycin only. The multidrug-resistant *Acinetobacter* was identified as *A. baumannii* by AFLP because the isolates clustered with the reference strain of *A. baumannii* 50% or more. The AFLP profiles of patient and environmental isolates clustered well above 90%, indicating that they belonged to the same strain (Figure). After removal of the dust from the interior of the ventilator by forced air, the outbreak strain was no longer isolated from the machine.

The second outbreak was caused by an *Acinetobacter* resistant to all beta-lactam agents tested, the fluoroquinolones, and the aminoglycosides. The isolate was susceptible to meropenem only.

Different ventilators had been used for the affected patients. The only pieces of equipment that had been used by all colonized patients except one were the continuous veno-venous hemofiltration (CVVH) machines. During the 3-month period from the day of admission of the index patient to the day the CVVH machines were sampled, 175 patients were admitted to the medical ICU and the neurosurgery ICU. Eleven of these 175 patients received CVVH. The outbreak strain was isolated from 3 (27%) of the 11 patients, and 2 (1%) of 164 patients not receiving CVVH were found to harbor the outbreak strain. Thus, patients who had the outbreak strain were more likely to have received CVVH (odds ratio, 30.4; 95% confidence interval, 6 to 146). The outbreak strain was isolated from dust inside both the blood compartment and the substitution compartment of 4 of the 6 machines present in our hospital. The isolates of both patients and CVVH machines were identified as *A. baumannii* and appeared to represent the same strain in AFLP analysis (Figure). After removal of the dust from the interior of all CVVH machines, the outbreak strain was no longer isolated from any of the machines or patients.

DISCUSSION

Bacteria belonging to the genus *Acinetobacter* are known to be capable of surviving in dry conditions.[9,10]

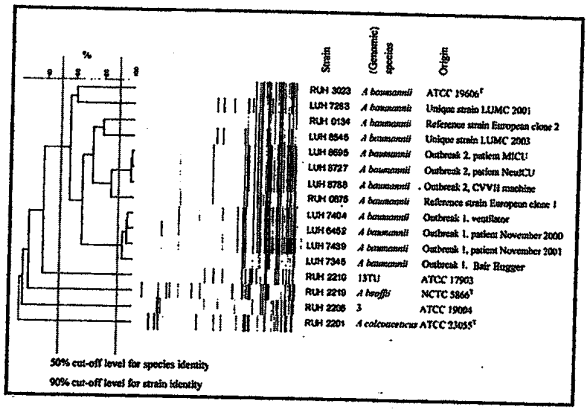

FIGURE. Amplified fragment-length polymorphism fingerprints of patients' isolates, of isolates from medical equipment of the first outbreak (outbreak 1) and the second outbreak (outbreak 2), and of reference strains of five *Acinetobacter* species, including *A. baumannii*. Levels of similarity are expressed as percentages of similarity. RUH = Rotterdam University Hospital; LUH = Leiden University Hospital; LUMC = Leiden University Medical Center; MICU = medical intensive care unit; NeuICU = neurosurgery ICU; CVVH = continuous veno-venous hemofiltration; ATCC = American Type Culture Collection; NCTC = National Collection of Type Cultures.

Dust contaminated with *A. baumannii* may thus be a relevant vehicle in the transmission of this bacterium.

In our 882-bed, tertiary-care hospital, *A. baumannii* is not a frequent occurrence. The numbers of patients from whom *A. baumannii* was isolated from 1999 through 2002 were 5, 23, 29, and 5, respectively, with incidences of 0.03, 0.07, 0.06, and 0.03 per 1,000 patient-days, respectively. Ten of the 23 *A. baumannii* isolates in 2000 and 19 of the 29 in 2001 were the outbreak strain and occurred in the ICUs only.

In the first outbreak, the strain involved was found in the interior of a ventilator and of the Bair Hugger. After removal of the dust inside all ventilators and replacement of the filters of the Bair Hugger, the outbreak strain was no longer isolated from patients. In the second outbreak, the strain involved was isolated from dust inside the CVVH machines, which had been used on all colonized patients except one. After removal of the dust from the CVVH machines using forced air, the outbreak strain was no longer isolated.

During operation, a fan provides continuous airflow through ventilators and CVVH machines to cool the circuit boards. It is possible that dust carrying bacteria is passed in and out of the machines on this air current, despite dust filters being placed at the air inlets and outlets. The Bair Hugger is designed to create an airflow; dust is sucked into the machine, with filters becoming contaminated and possibly serving as a secondary source of transmission. It was not known how long the filters had been in place, and there was no protocol for regular replacement of the filters. We believe the outbreak strain was transmitted by being carried on contaminated dust from within the machines to the exterior during operation when a fan created an air current. Thus, the exterior of

the machines may have been contaminated and become a secondary source of spread.

We found contaminated dust in the interior of different types of machines used by patients on two different occasions. After this dust was removed, no further cases were observed. Hence, dust may be relevant in the epidemiology of *A. baumannii* or of any microorganism capable of surviving under dry conditions. We recommend that during outbreaks of *A. baumannii* the removal of dust from the interior of machines that patients come in close contact with be an integral part of cleaning and disinfection procedures.

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Epidemiologic Study of Nosocomial Urinary Tract Infections in Saudi Military Hospitals

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ABSTRACT

A case–control study of patients with and without confirmed UTI was performed to identify risk factors for nosocomial UTI. Duration of hospitalization, unit of admission, history of diabetes mellitus or debilitating diseases, and duration and number of urinary catheters were independently associated with increased risk of nosocomial UTIs (*Infect Control Hosp Epidemiol* 2004;25:1004-1007).

In developing countries, nosocomial infection is increasingly being recognized as a significant problem. Nosocomial infection often results in extended hospitalization, expensive therapy, and morbidity and mortality.[1,2] Up to 10% of all hospitalized patients develop nosocomial infection.[3,4] Urinary tract infections (UTIs) are the most common type of nosocomial infection, accounting for 40% of all infections in hospitals and 34% of all infections in nursing homes.[5,6] In hospitals, 80% to 90% of nosocomial UTIs are associated with the use of urinary catheters and an additional 5% to 10% are associated with other genitourinary manipulations.[6-8] Prevention and management of such infections require an intimate knowledge of their epidemiology, including risk factors.[9,10] Hospital infection control programs can prevent 33% of nosocomial infections, including nosocomial UTIs.[11] The aims of this study were to estimate the overall rates of nosocomial infections and nosocomial UTIs and their linear trends during 5 years (1998 to 2002) and to identify potential risk factors of hospitalized patients who developed nosocomial UTIs.

METHODS

To fulfill the objectives of this study, two methodologies were adopted: a case–control study of risk factors and a record review to calculate nosocomial UTI rates.

The case–control study of risk factors was performed between August 1, 2001, and July 31, 2003, at Al-Hada (400 beds), Al-Rehab (100 beds), and Prince Sultan (50 beds) military hospitals. These three hospitals are administered by the Medical Service Department of the Saudi Arabian Ministry of Defense and Aviation. All patients admitted to these hospitals for at least 72 hours during the study period were considered eligible for inclusion in the study. Among these, patients proved to have UTI were considered case-patients. The diagnosis of UTI was made according to criteria of the Centers for Disease Control and Prevention.[12] After exclusion of patients who did not fulfill eligibility criteria, three control-patients were enrolled for each case-patient through a systematic random sampling procedure using the patient admission record list (every three patients).

For all participants (case-patients and control-patients), the following were recorded: age, gender, unit of admission, presence of a catheter, duration of catheterization, number of catheters, history of diabetes mellitus, history of immunosuppressive drug use, history of debilitating diseases (cancer, liver failure, or uremia), and duration of hospitalization. These data were collected from the patients' records during their hospital stay by a trained nosocomial infection surveillance team from the Preventive Medicine Department.

Hospital records, providing the number of patients hospitalized each month and the numbers of nosocomial infections (crude and site specific) each month, were reviewed. The overall annual rates of nosocomial infections and nosocomial UTIs during the period 1998 to 2002 were calculated by dividing the total number of nosocomial infections (crude and UTIs) pooled throughout all